

## Interactions of SKIP/NCoA-62, TFIIB, and Retinoid X Receptor with Vitamin D Receptor Helix H10 Residues\*

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**The vitamin D receptor (VDR) is a ligand-dependent transcription factor that heterodimerizes with retinoid X receptor (RXR) and interacts with the basal transcription machinery and transcriptional cofactors to regulate target gene activity. The p160 coactivator GRIP1 and the distinct coregulator Ski-interacting protein (SKIP)/NCoA-62 synergistically enhance ligand-dependent VDR transcriptional activity. Both coregulators bind directly to and form a ternary complex with VDR, with GRIP1 contacting the activation function-2 (AF-2) domain and SKIP/NCoA-62 interacting through an AF-2 independent interface. It was previously reported that SKIP/NCoA-62 interaction with VDR was independent of the heterodimerization interface (specifically, helices H10/H11). In contrast, the present study defines specific residues within a conserved and surface-exposed region of VDR helix H10 that are required for interaction with SKIP/NCoA-62 and for full ligand-dependent transactivation activity. SKIP/NCoA-62, the basal transcription factor TFIIB, and RXR all interacted with VDR helix H10 mutants at reduced levels compared with wild type in the absence of ligand and exhibited different degrees of increased interaction upon ligand addition. Thus, SKIP/NCoA-62 interacts with VDR at a highly conserved region not previously associated with coregulator binding to regulate transactivation by a molecular mechanism distinct from that of p160 coactivators.**

The vitamin D receptor (VDR)<sup>1</sup> is a ligand-dependent transcription factor important in the regulation of calcium homeo-

stasis, development, cell growth, and differentiation. VDR belongs to the nuclear receptor superfamily, members of which share a common modular structure including a highly conserved DNA binding domain (DBD) and a conserved ligand binding domain (LBD). The LBD has a predominantly  $\alpha$ -helical structure with an activation function-2 (AF-2) domain in the COOH-terminal helix (H12). The LBD is the main site of VDR interaction with its heterodimer partner retinoid X receptor (RXR) and basal transcription factors, and the AF-2 domain in combination with the hydrophobic cleft forms an interaction surface for transcriptional corepressors and coactivators (1, 2). In the absence of its ligand 1,25-dihydroxyvitamin D<sub>3</sub> (1,25(OH)<sub>2</sub>D<sub>3</sub>), VDR transcriptional activity is low due to interaction with corepressors such as N-CoR and SMRT and potentially through sequestration of the basal transcription factor TFIIB. Addition of 1,25(OH)<sub>2</sub>D<sub>3</sub> causes dissociation of VDR-corepressor and VDR-TFIIB complexes, thereby relieving repression (3–5). VDR ligand-dependent activation is facilitated by recruitment of at least one coactivator protein with intrinsic histone acetyl transferase activity, such as p160 coactivators. Recruitment of multiprotein cointegrator complexes including CBP/p300, p/CAF, and DRIP provide further regulation, coordinating interactions of the receptor with other proteins including basal transcription factors, RNA polymerase II, and the p160 coactivators (6).

The novel coregulator Ski-interacting protein (SKIP)/NCoA-62, which is structurally distinct from the p160 coactivators, was isolated through its interactions with both the Ski oncoprotein and VDR (7, 8). It also interacts with other nuclear proteins including the repressor CBF-1 and the HPV-16 E7 oncoprotein, can bind coregulators such as SMRT, Sin3A, HDAC2, and SRC-1, and interacts with Smad proteins to augment transforming growth factor- $\beta$ -dependent transactivation (7–12). SKIP/NCoA-62 functions in cell fate determination, alternatively facilitating gene repression by tethering CBF-1 to the corepressor SMRT and gene activation by tethering CBF-1 to the activator Notch1C (10). Such mutually exclusive interactions with coactivators and corepressors suggest that SKIP/NCoA-62 may facilitate coregulator switching.

SKIP/NCoA-62 augments transactivation by the vitamin D, retinoic acid, estrogen, and glucocorticoid receptors (7), selectively binding the VDR:RXR heterodimer in a ligand-enhanced manner (13). SKIP/NCoA-62 forms a ternary complex and acts synergistically with the p160 coactivator GRIP1 to augment VDR transactivation (13). Consistent with such complex formation, p160 coactivators contact VDR through the AF-2 do-

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<sup>1</sup> The abbreviations used are: VDR, vitamin D receptor; RXR, retinoid X receptor; SKIP, Ski-interacting protein; DBD, DNA binding domain;

LBD, ligand binding domain; AF-2, activation function 2; 1,25(OH)<sub>2</sub>D<sub>3</sub>, 1,25-dihydroxyvitamin D<sub>3</sub>; RAR, retinoic acid receptor; PPAR, peroxisome proliferator-activated receptor; HA, hemagglutinin; GST, glutathione S-transferase.

main, whereas SKIP/NCoA-62 interactions with VDR are AF-2-independent. SKIP/NCoA-62 reportedly contacts helix H1 of the VDR LBD (residues 116–164 of human VDR) and the helix H10/H11 region (residues 373–403) but not helix H12 (7). It was subsequently shown, however, that SKIP/NCoA-62 could bind to the VDR:RXR heterodimer, leading to the conclusion that the heterodimerization interface (including helix H10/H11) could be excluded as a SKIP/NCoA-62 interaction surface (13).

In the present study, we further investigated structural aspects of VDR-SKIP/NCoA-62 interaction to determine how structure may mediate SKIP/NCoA-62 function. An interaction site was mapped to helix H10 of mouse VDR by mutational analysis. Alteration of structurally exposed amino acids of this helix markedly reduced 1,25(OH)<sub>2</sub>D<sub>3</sub>-independent interactions with SKIP/NCoA-62 but not ligand-dependent interactions with the p160 coactivators GRIP1 and RAC3. These same VDR residues were required for interaction with RXR regardless of ligand status and for ligand-independent interaction with TFIIB. The results indicate that a transcriptional coactivator, a basal transcription factor, and the VDR heterodimerization partner each interact with VDR at the same helix H10 interface, with varying ligand sensitivities. As SKIP/NCoA-62 interacts with both unliganded and liganded VDR, the data also suggest that it may modulate the ligand-regulated occupancy of this VDR surface by TFIIB and RXR.

#### EXPERIMENTAL PROCEDURES

**Plasmids and Mutagenesis**—Mouse VDR (mVDR) was amplified from full-length cDNA (T. Kawada, Kyoto University) using wild type or mutant primers containing stop codons (for truncation mutants Δ397, Δ389, and Δ380) or point mutations and cloned into pSG5 (Stratagene) and pAS2-1 (Clontech). Mutant Δ389 was further mutated to create Δ389Q380E, Δ389D384K, Δ389R386E, and Δ389AX3, the last being a triple point mutant with coordinate Q380A, D384A, and R386A changes. Full-length wild type cDNA was similarly mutated to produce mutant AX3. Constructs pACTIISKIP (amino acids 145–536) (12), pACTIITFIIB (14), pACTIIβ-RXRα, pGAD424-GRIP1 (nucleotides 204–4878), pGAD10-RAC3 (nucleotides 1289–3698), pSG5GRIP1, and pSG5RAC3 (15) have been described.

**Molecular Modeling**—A model of the mouse VDR LBD was built using the 1.8-Å resolution crystal structure of the liganded human VDR LBD as a template (Protein Data Bank accession code 1DB1; Ref. 2). Initial CLUSTALW (16) alignments were performed using the LBD sequences of hRARα, -β, and -γ; hRXRα, -β, and -γ; hTRα and -β; hPPARα, -β, and -γ; and hVDR, rVDR, and mVDR, with subsequent analyses performed using hRARγ, hVDR, rVDR, and mVDR sequences. Modeling was performed using InsightII and Homology version 98 software (Molecular Simulations Inc., San Diego, CA). The model of the LBD was built from residue 124 and required no insertions or deletions. Side chain rotamer positions were searched by retaining identical residues in their original positions, and automatically placing others based on their size and flexibility, placing large hydrophobic residues before small side chains. Species-related changes in the structure of mVDR due to the excised insertion domain or sequence differences, which are away from the ligand binding and interaction sites, are anticipated to be minor.

**Yeast Two-hybrid Assays**—pAS2-1mVDRWT or mutant constructs were transformed into SFY526 cells and tryptophan-selected. pACTIISKIP (amino acids 145–536), pACTIITFIIB, pACTIIβ-RXRα, pGAD424-GRIP1, and pGAD10-RAC3 were individually transformed into Y187 cells and leucine-selected. Haploid SFY526 and Y187 strains were mated and diploid strains selected with tryptophan and leucine. Six independent colonies from each diploid strain were tested in ligand assays as described previously (14). Cell lysate protein content was determined (Bio-Rad), and β-galactosidase activity was assayed by chemiluminescence (Tropix).

**Immunoblot Analysis**—Lysates were prepared from diploid yeast strains (14) and mammalian cells (17). Equal amounts of protein were resolved by SDS-PAGE (10%), blotted, and probed with 9A7 antibody (Affinity Bioreagents Inc.) to detect VDR and anti-HA tag antibody (Roche Molecular Biochemicals) to detect HA-SKIP.

**GST Binding Assays**—GST-SKIP (12), GST-TFIIB (18), GST-

mRXRβ (19), and GST-O (Amersham Biosciences) fusion proteins were expressed in *Escherichia coli* BL21, purified, and coupled to glutathione-Sepharose as described (Amersham Biosciences). Whole cell lysates containing VDR wild type and mutant proteins were prepared (20) from COS-1 cells transfected with pSG5 mVDR expression constructs. GST binding reactions were performed (14) in the presence of 10<sup>-7</sup> M 1,25(OH)<sub>2</sub>D<sub>3</sub> or vehicle. Bound proteins were eluted, electrophoresed, and immunoblotted with 9A7 antibody.

**Coimmunoprecipitation Assays**—pSG5WT and mutant VDR constructs, pSG5GRIP1 and pSG5RAC3, were transcribed and translated *in vitro* using the TNT<sup>TM</sup> T7 quick coupled system (Promega). [<sup>35</sup>S]Methionine was incorporated into GRIP1 and RAC3 polypeptide products. Binding reactions were performed (21) in the presence of 10<sup>-7</sup> M 1,25(OH)<sub>2</sub>D<sub>3</sub> or vehicle. Complexes were immunoprecipitated with 9A7 antibody and anti-rat IgG-Sepharose 4B beads (Zymed Laboratories Inc.). Bound complexes were eluted, separated by SDS-PAGE (10%), and dried gels were autoradiographed.

**Cell Culture and Transfections**—COS-1 cells were transfected for 6 h using FuGENE<sup>TM</sup> (Roche Molecular Biochemicals) with 30 ng of pSG5WT or mutant, 450 ng of pGL3-24-hydroxylase-LUC reporter (3), and 60 ng of pRSVβgal control plasmid. Cells were then treated with vehicle or 10<sup>-10</sup>, 10<sup>-8</sup>, or 10<sup>-6</sup> M 1,25(OH)<sub>2</sub>D<sub>3</sub> for 16 h, harvested, and assayed for luciferase (Promega) and β-galactosidase activities. Luciferase activity was corrected for transfection efficiency using β-galactosidase results.

#### RESULTS

**VDR Helix H10 Residues Form SKIP/NCoA-62 Interaction Interface**—SKIP/NCoA-62 has been reported to interact with human VDR through residues 373–403 in the helix H10/H11 region, excluding the AF-2 domain (7). In the present study a series of truncation mutants in this region of the highly conserved mouse VDR were generated, deleting helices H12 (Δ397), H11, and H12 (Δ389) or part of H10 plus H11 and H12 (Δ380), the last mutation removing the highly conserved ninth heptad repeat (1) (Fig. 1, A and B). Wild type VDR interacted with SKIP/NCoA-62 in the absence and presence of ligand in yeast two-hybrid assays, as did the Δ397 and Δ389 mutants (Fig. 2A). The Δ380 mutant failed to interact with SKIP/NCoA-62, implicating mVDR residues 380–389 in this interaction.

A model of the mVDR LBD was built based on the highly homologous (89% identity) human VDR LBD (2). Residues 380–389 lie on helix H10 at the receptor surface (Fig. 1C), forming part of an exposed ridge, with the polar or charged side chains of the conserved residues Gln-380, Asp-384, and Arg-386 particularly prominent midway along the helix. The guanidinium group of Arg-386 appears in a highly conserved bridge with Asp-337, stacked on Arg-338, making the aliphatic component of the side chain more prominent in the model structure. Based on the accessibility of the side chains to the exterior, flexibility in Gln-380, Asp-384, and Arg-386 is anticipated. To investigate their potential involvement in SKIP/NCoA-62 interaction, these three residues were individually mutated to retain similar bulk but altered charge in the context of Δ389 (Q380E, D384K, or R386E; Fig. 1A). Each point mutation diminished VDR interaction with SKIP/NCoA-62 by 60–80% compared with wild type and Δ389 controls (Fig. 2B). Similarly, coordinate mutation of these residues to alanine in the Δ389 context (Δ389AX3 mutant) decreased this interaction by 80%. Interaction of the full-length VDR AX3 mutant was limited to ~25% of wild type in the absence of ligand, but approached wild type levels with 1,25(OH)<sub>2</sub>D<sub>3</sub> (Fig. 2C). Wild type VDR interaction with GST-SKIP *in vitro* was also comparable both with and without ligand (Fig. 2D). Mutant AX3 bound less strongly, although without the ligand-induced increase in SKIP/NCoA-62 interaction seen in yeast.

**VDR Helix H10 Not Required for p160 Coactivator Interaction**—In contrast to SKIP/NCoA-62, interactions of the p160 coactivators GRIP1 and RAC3 with wild type and AX3 mutant VDR were entirely ligand-dependent (Fig. 3A). Interestingly,



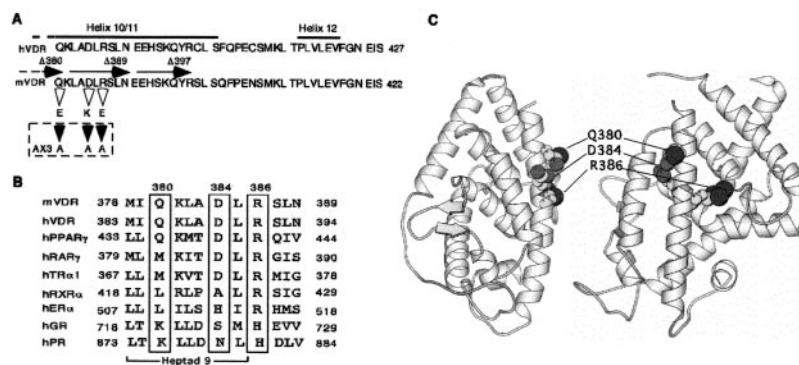


FIG. 1. VDR COOH-terminal mutant structure and LBD modeling. *A*, alignment of human and mouse VDR COOH-terminal amino acid sequences.  $\alpha$ -Helical regions are indicated by *overlines*. Truncation points of mutants  $\Delta$ 397,  $\Delta$ 389, and  $\Delta$ 380 are indicated by *horizontal arrows*. Point mutations indicated by *unfilled vertical arrows*; coordinate mutation of residues 380, 384, and 386 in AX3 mutant is indicated by *filled vertical arrows* enclosed in a *dashed box*. *B*, alignment of helix H10 amino acid residues. Residues analogous to mVDR Gln-380, Asp-384, and Arg-386 are *boxed*. Residues within the ninth heptad repeat are indicated. *C*, ribbon diagrams of mVDR LBD model. *Left panel* shows the prominent helix H10 residues Gln-380, Asp-384, and Arg-386 in profile, with the helix oriented so that the right side represents the most exposed region. The *right panel* is rotated 90 degrees clockwise. The figure was prepared with MOLSCRIPT (31).

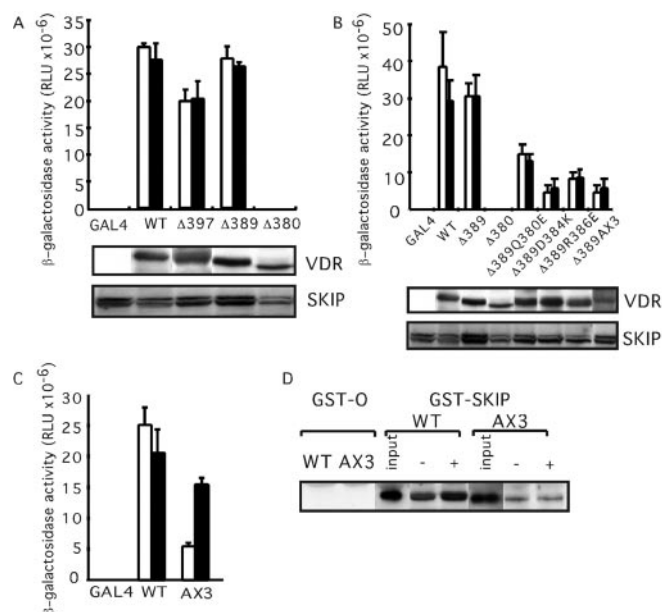


FIG. 2. SKIP/NCoA-62 interactions with VDR COOH-terminal mutants. Yeast two-hybrid assay of strains expressing HA-tagged SKIP (amino acids 145–536), with mVDR wild type and mutants: *A*,  $\Delta$ 397,  $\Delta$ 389, or  $\Delta$ 380; *B*,  $\Delta$ 389,  $\Delta$ 389Q380E,  $\Delta$ 389D384K,  $\Delta$ 389R386E, and  $\Delta$ 389AX3; *C*, AX3 and treated with vehicle (*open bars*) or  $10^{-8}$  M  $1,25(\text{OH})_2\text{D}_3$  (*solid bars*). Western blots confirm VDR and HA-SKIP expression. *D*, *in vitro* binding assay of mVDR wild type or AX3 with GST-SKIP or GST-O, with or without  $10^{-7}$  M  $1,25(\text{OH})_2\text{D}_3$ . Input was 10% of VDR added. Electrophoresed products were probed for VDR protein levels.

p160 coactivator interactions with AX3 were elevated by  $\sim 50\%$ , suggesting that residues Gln-380, Asp-384, and Arg-386 may negatively affect access of p160 coactivators to the hydrophobic cleft on the LBD. Neither  $\Delta$ 389 nor  $\Delta$ 389AX3 interacted with GRIP1 or RAC3, consistent with the AF-2 deletion. *In vitro* interactions between the p160 coactivators and VDR mutants were consistent with the yeast data (Fig. 3*B*). Thus, VDR helix H10 is important for interaction with SKIP/NCoA-62 but not p160 coactivators.

**VDR Helix H10 Mutations Inhibit Transactivation Function**—Effects of these helix H10 mutations on VDR function were assessed by transient transfection. Transactivation by either wild type or AX3 mutant VDR was  $1,25(\text{OH})_2\text{D}_3$  dose-responsive, but AX3 activity was limited to about 40% of wild type at the highest dose tested, despite comparable VDR pro-

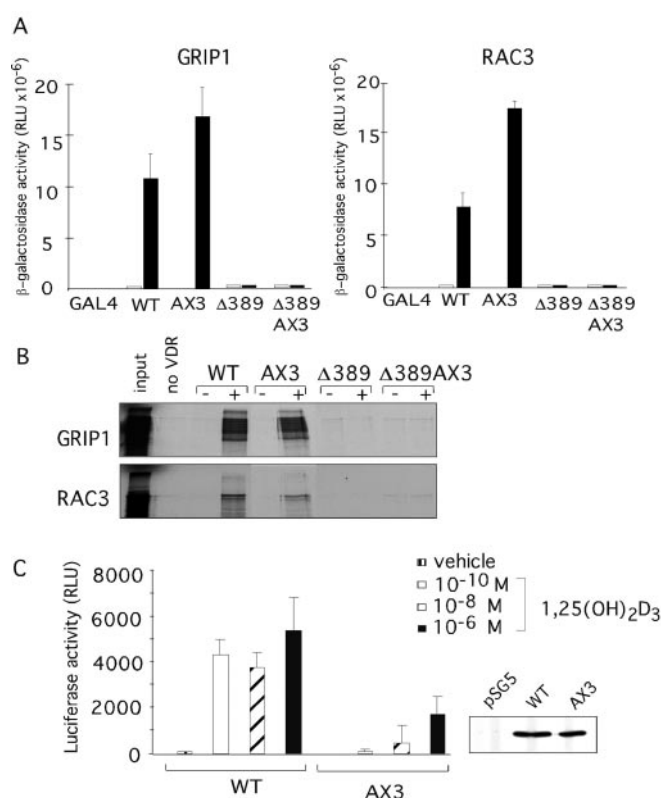
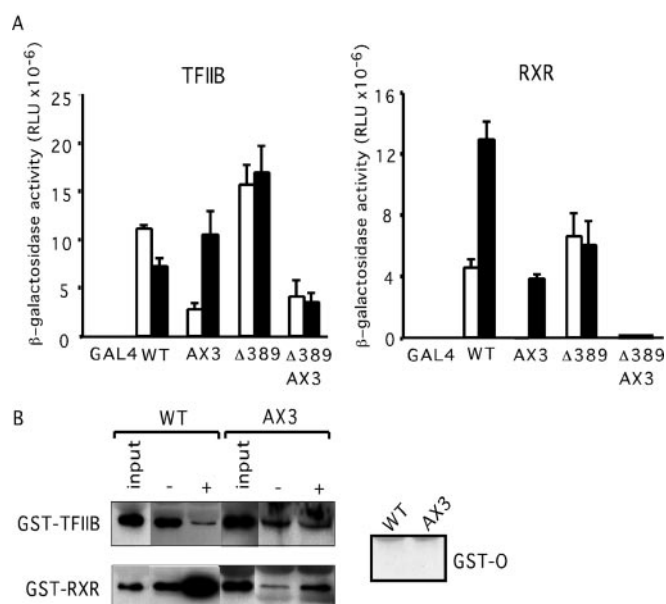


FIG. 3. VDR H10 mutant interactions with p160 coactivators and transactivation function. *A*, yeast two-hybrid interactions of mVDR wild type, AX3,  $\Delta$ 389, or  $\Delta$ 389AX3 mutant, and GRIP1 or RAC3. Treatment with vehicle (*open bars*) or  $10^{-8}$  M  $1,25(\text{OH})_2\text{D}_3$  (*solid bars*). *B*, immunoprecipitations of *in vitro* translated VDR and GRIP1 or RAC3 ( $^{35}\text{S}$ -labeled). Complexes were precipitated using the VDR-specific 9A7 antibody. Input was 10% of coactivator added; “no VDR” was coactivator immunoprecipitated without VDR. *C*, AX3 mutant transactivation function. Wild type or AX3 mutant VDR was transfected into COS-1 cells with the 24-hydroxylase promoter-luciferase reporter and treated with increasing concentrations of  $1,25(\text{OH})_2\text{D}_3$ . VDR expression in transfected lysates was confirmed by immunoblotting.

tein levels (Fig. 3*C*). Thus, the integrity of helix H10 residues Gln-380, Asp-384, and Arg-386 is necessary for efficient VDR transactivation.

**Other Interactions at Helix H10 Interface**—In addition to SKIP/NCoA-62, TFIIB and RXR also interact with this region of VDR (5, 22–24), so their interactions with the helix H10 mutants were investigated. Two-hybrid interaction of wild type



**FIG. 4. VDR H10 mutant interactions with TFIIB and RXR.** *A*, yeast two-hybrid interactions of mVDR wild type, AX3,  $\Delta$ 389, or  $\Delta$ 389AX3 mutant with TFIIB or RXR, with vehicle (open bars) or  $10^{-8}$  M  $1,25(\text{OH})_2\text{D}_3$  (solid bars) treatment. *B*, *in vitro* binding of mVDR wild type or AX3 with GST-TFIIB, GST-RXR, or GST-0, with or without  $10^{-7}$  M  $1,25(\text{OH})_2\text{D}_3$ . Input was 10% of VDR used in binding reactions. Electrophoresed products were probed for VDR protein levels.

VDR with TFIIB was inhibited by 30% upon addition of  $1,25(\text{OH})_2\text{D}_3$  (Fig. 4A), consistent with a previous report that VDR-TFIIB contact is inhibited by ligand (5). Interaction of AX3 with TFIIB in the absence of ligand was  $\sim 25\%$  of wild type, but reinstated to wild type level with  $1,25(\text{OH})_2\text{D}_3$ . Interaction of  $\Delta$ 389 with TFIIB was independent of ligand and at least as strong as wild type. In contrast, interaction of  $\Delta$ 389AX3 with TFIIB was notably diminished, with and without ligand. Binding of wild type VDR with GST-TFIIB *in vitro* was also inhibited by ligand (Fig. 4B). AX3-TFIIB interaction was weaker than wildtype, but with no ligand-induced increase as in yeast.

Unliganded wild type VDR interacted with RXR and was augmented  $\sim 3$ -fold by  $1,25(\text{OH})_2\text{D}_3$  (Fig. 4A). In contrast, unliganded AX3 did not interact with RXR, and  $1,25(\text{OH})_2\text{D}_3$  induced interaction to only 30% of wild type level. Mutant  $\Delta$ 389-RXR interaction was comparable with wild type VDR without ligand and was unaffected by ligand treatment, as  $\Delta$ 389 does not bind  $1,25(\text{OH})_2\text{D}_3$  (data not shown). Coordinate alanine mutation completely abrogated  $\Delta$ 389AX3 interactions with RXR. *In vitro* binding data of VDR with RXR correlated with yeast results, as binding of wild type and AX3 to GST-RXR increased with ligand, with AX3 binding lower than wild type (Fig. 4B). Together these data indicate that mVDR helix H10 residues 380, 384, and 386 are important for interactions with TFIIB and RXR as well as SKIP/NCoA-62. Ligand effects on these interactions of wild type VDR differed, as VDR-SKIP/NCoA-62 interactions were ligand-independent, whereas VDR-TFIIB interactions were inhibited by  $1,25(\text{OH})_2\text{D}_3$  and those for VDR:RXR were augmented.

#### DISCUSSION

SKIP/NCoA-62 is unique among nuclear receptor coregulators in that its interactions with VDR appear to be independent of the hydrophobic cleft/AF-2 domain interface, relying instead on an undefined LBD site. In this study we defined the structural nature of this interaction interface and highlighted its importance in VDR function and protein interactions. COOH-

terminal mutagenesis of mVDR identified three helix H10 residues, Gln-380, Asp-384, and Arg-386, required for full interaction with SKIP/NCoA-62, but not with p160 coactivators. Distinct differences in ligand-responsive interactions between helix H10 mutants and SKIP/NCoA-62, TFIIB, or RXR were observed. Helix H10 mutation also attenuated VDR transactivation function, presumably as a result of disrupted interactions not only with TFIIB and RXR, but also with SKIP/NCoA-62. Wild type VDR interacted with SKIP/NCoA-62 in both the absence and presence of  $1,25(\text{OH})_2\text{D}_3$ , whereas its interaction with TFIIB was partially inhibited and with RXR was augmented by  $1,25(\text{OH})_2\text{D}_3$ , consistent with earlier reports (5, 7, 13).

Mutagenesis of mVDR H10 residues Gln-380, Asp-384, and Arg-386 markedly reduced ligand-independent interactions with SKIP/NCoA-62 and TFIIB and completely abrogated RXR interaction. Ligand treatment restored interactions with TFIIB fully, and with SKIP/NCoA-62 and RXR partially. Thus, in the absence of ligand the bulky side chains of these helix H10 residues appear to present a prominent interaction site, as their removal in AX3 attenuated protein-protein interactions. The reinstatement of interactions upon ligand treatment may result from a conformational change that brings additional interacting regions into play. This proposal is consistent with a requirement for hVDR helix H1 residues 116–165 for SKIP/NCoA-62 interaction (7), and for sites in the DNA binding and ligand binding domains of VDR for TFIIB and RXR interactions (22–26).

Interaction of SKIP/NCoA-62 with the VDR helix H10 interface would be compatible with concurrent binding of GRIP-1 to the AF-2-dependent hydrophobic cleft and thus consistent with ternary complex formation in the presence of ligand (13). SKIP/NCoA-62 interaction with helix H10 of unliganded VDR would also be compatible with formation of a repressive ternary complex in the absence of ligand, as SKIP/NCoA-62 and unliganded VDR both interact with NCoR and SMRT (3, 4, 10). Furthermore, by analogy with its tethering function in the alternate recruitment of corepressors and coactivators in Notch pathway regulation (10), SKIP/NCoA-62 may perform a similar function in VDR signaling, *i.e.* coordinating interactions with unliganded VDR to mediate transcriptional repression and with liganded receptor to mediate a switch to transcriptional activation. This proposal is supported by the finding that SKIP/NCoA-62 can augment or repress VDR transactivation in a cell-specific manner (27).

Modulation of VDR protein interactions by SKIP/NCoA-62 may also help regulate its interactions with TFIIB and RXR. It has been proposed that ligand-induced disruption of VDR-TFIIB interactions releases TFIIB for incorporation into the transcription complex (5). SKIP/NCoA-62 may regulate this transition through dynamic helix H10 interactions, as it can interact directly with TFIIB (data not shown) in addition to both unliganded and liganded VDR. Similarly, SKIP/NCoA-62 may facilitate RXR interaction with VDR when ligand is present, consistent with formation of a ternary complex between SKIP/NCoA-62 and the liganded VDR:RXR heterodimer (13).

Such a modulator role for SKIP/NCoA-62 may also be important for nuclear receptors other than VDR. Residues Gln-380, Asp-384, and Arg-386 are in the ninth heptad repeat region, which is highly conserved among the nuclear receptors. Consistent with this conservation, TFIIB interacts with RXR $\beta$  and TR $\beta$  (14, 28), and RXR $\alpha$  contacts PPAR $\gamma$  and RAR $\alpha$  (29, 30) through heptad 9 residues analogous to mVDR Gln-380, Asp-384, and Arg-386. By extension, therefore, SKIP/NCoA-62 may also interact with nuclear receptors other than VDR via the helix H10 interface.

In summary we have found that the coregulator SKIP/NCoA-62 interacts with VDR via a helix H10 interface that is functionally and physically distinct from the region bound by the AF-2-dependent p160 coactivators. This previously unidentified coactivator interaction surface is required for full VDR transactivation activity and comprises conserved residues from helix H10 that are also involved in TFIIB interaction and heterodimerization with RXR. Moreover, the interactions of SKIP/NCoA-62, TFIIB, and RXR with the VDR helix H10 interface are dynamic, with varying responses to ligand. This study elucidates the structural basis of VDR-SKIP/NCoA-62 interactions and suggests that SKIP/NCoA-62 may regulate the exchange between TFIIB and RXR at the VDR helix H10 interface. Furthermore, it highlights the novel role of SKIP/NCoA-62 in VDR transcriptional regulation, a role that may also extend to other nuclear receptors.

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**Interactions of SKIP/NCoA-62, TFIIIB, and Retinoid X Receptor with Vitamin D Receptor Helix H10 Residues**

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